Experimental (Antiestrogen-Mediated) Reduction in Egg Size Negatively Affects Offspring Growth and Survival

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ABSTRACT

The relationship between egg size and offspring phenotype is critical to our understanding of the selective pressures acting on the key reproductive life-history traits of egg size and number. Yet there is surprisingly little empirical evidence to support a strong, positive relationship between egg size and offspring quality (i.e., offspring growth, condition, and survival) in birds,

Figure 1. Relationship between mean egg mass (g) and clutch size in tamoxifen-treated females (*open circles, dashed line*) and sham-treated females (*filled circles, solid line*).

on at 0700 hours). The birds received a mixed-seed diet (panicum and white millet 1 : 1; approximately 11.7% protein, 0.6% lipid, and 84.3% carbohydrate), water, grit, and cuttlefish bone (calcium) ad lib., and a multivitamin supplement in their drinking water once per week. All experiments and animal husbandry were carried out under a Simon Fraser University Animal Care Committee permit (692B-94) following guidelines of the Canadian Committee on Animal Care.

Before the experiment, all birds were housed in same-sex cages (61 cm × 46 cm × 41 cm) but were not visually or acoustically isolated from the opposite sex. The females selected for this experiment were 6–18 mo in age and had been successfully bred once or twice, allowing for repeated-measures analyses of primary reproductive traits (i.e., pretreatment mean egg mass,

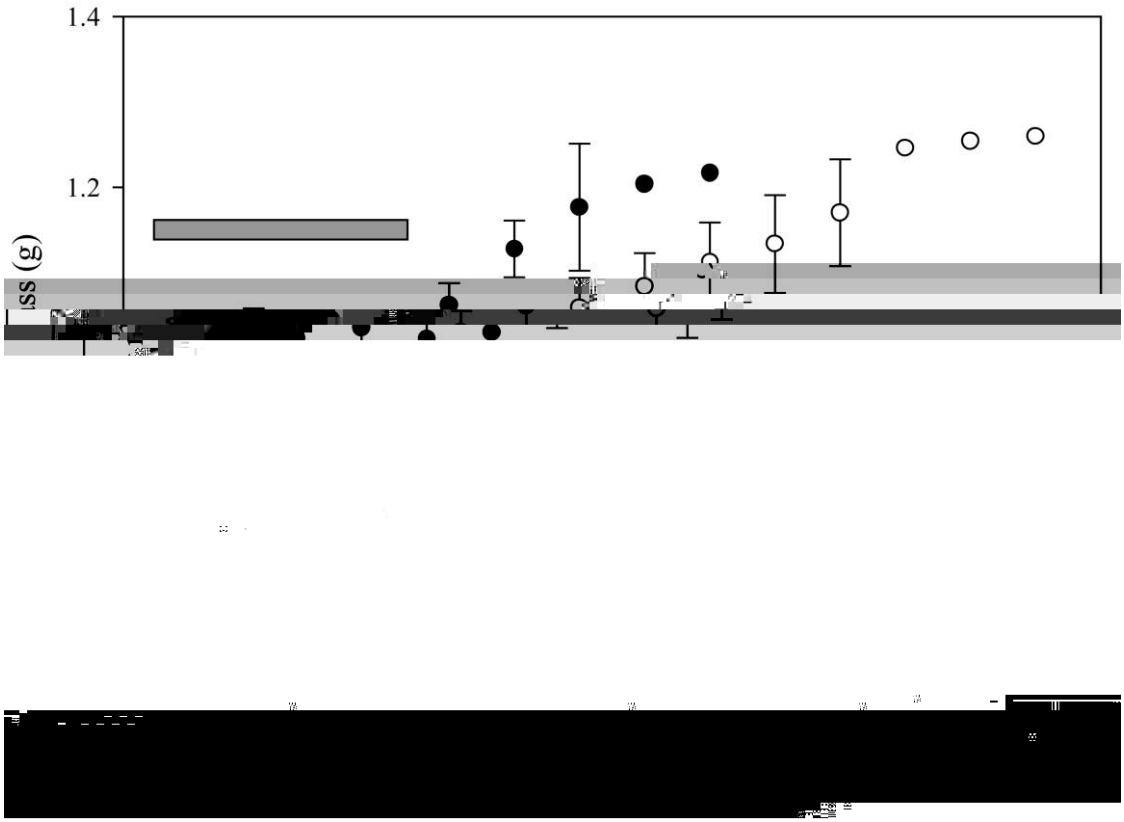

Figure 2. Variation in egg mass (g) with laying sequence in tamoxifen-treated females (*open circles*) and sham-treated females (*filled circles*); the shaded bar indicates the treatment period. Values are least squares means ± SEM. In the sham treatment group, only one female laid nine eggs, and in the tamoxifen treatment group, only one female laid 14 eggs.

Experimental Protocol

Initially we had designed this experiment to further address the effect of short-term tamoxifen treatment on the trade-off between egg size and clutch size (Williams 2001). In the zebra finch, clutch size is believed to be determined between deposition of the third and fourth eggs (Haywood 1993), and to target this critical period we assigned females to three treatment groups, with cessation of tamoxifen injections at the two-egg (n p 17), three-egg (n p 17), and four-egg (n p 18) stages. However, in preliminary analysis we found no significant difference in clutch size or clutch mass between treatment groups and the control group (clutch size: $F_{4,67}$ p 1.07, P 1 0.15; clutch mass: $F_{3,71}$ p 1.02, P 1 0.35), and while tamoxifen treatment decreased egg size in all three treatment groups, there was no significant difference in egg size between the tamoxifen-treated groups ($F_{3,48}$ p 1.51, P 1 0.2). Therefore, for this article we have pooled data for the two-egg, three-egg, and four-egg treatment groups into a single tamoxifen treatment group (n p 52), and we focus on the relationship between egg size and offspring growth and survival. Females were thus randomly assigned to two groups. (*a*) Tamoxifen-treated females (n p 52) received daily intramuscular (pectoral) injections of tamoxifen citrate (10 g g^{-1} body weight in 30 L 1,2-propanediol) until they laid their second, third, or fourth egg (females originally assigned to the two-egg and three-egg treatments received injections of 1,2-propanediol vehicle until the fourth egg had been laid to ensure that handling was consistent between individuals). (*b*) Sham-treated females (n p 22) received daily intramuscular injections of only 1,2-propanediol until the fourth egg had been laid. All injections were given between 0830 and

Table 2: Comparison of egg fates for sham-treated and tamoxifen-treated females

Egg Fate	Sham-Treated Females	Tamoxifen-Treated Females
n (females)	22	52
Number of eggs where fate known	100	291
Eggs hatched	46 (46.0%)	59 (20.3%)
Infertile eggs	32 (32.0%)	99 (34.0%)
Unhatched embryo	14 (14.0%)	82 (28.2%)
Missing	8 (8.0%)	51 (17.5%)

Note. Values are absolute counts followed by percentages in parentheses.

Statistical Analysis

All statistical analyses were carried out using SAS, version 9.1 (SAS Institute 2003). Treatment effects on reproductive traits were analyzed using generalized linear models (GLM procedure) to compare tamoxifen- and sham-treated birds, with covariates (e.g., pretreatment mean egg mass, pretreatment clutch size, treatment clutch size, and treatment mean egg mass) included as terms in the model where appropriate. Paired *t*-tests were used to compare differences between pretreatment and treatment reproductive traits within individual females. Treatment effects on egg hatchability, offspring mortality, and brood sex ratio were tested with generalized linear models (GENMOD procedure) in a two-level structure with individual offspring

Figure 3. Comparison of survival rates of offspring from tamoxifen-treated females (*open circles*) and sham-treated females (*filled circles*) from hatching to independence (30 d posthatch).

offspring that hatched but did not survive to fledging ($n = 13$; Table 5). Finally, females that showed the smallest relative decrease in egg mass (−8.8%) and laid a larger clutch (+2 eggs) produced at least one offspring that survived to fledging ($n = 9$; Table 5). Among sham-treated females, there was no relationship between mean egg size or clutch size and the reproductive outcome of a clutch ($P > 0.5$ in both cases).

Discussion

Tamoxifen is considered to be a pure antiestrogen in birds (e.g., Wilson and Cunningham 1981; Jaccoby et al. 1995), and the effects on egg size that we report here are probably mediated by tamoxifen binding to estrogen receptors in the liver and suppressing yolk precursor synthesis, consequently reducing circulating yolk precursor levels available for yolk formation (Williams 2000). Hepatic production of vitellogenin is primarily an estrogenic process (Capony and Williams 1981); however, the production of albumen proteins in the oviduct can also be secondarily stimulated by receptor-mediated binding of progesterones, androgens, and glucocorticosteroids in addition to estrogens (McKnight and Palmiter 1979), and the mechanisms of these hormones are unaffected (and in some cases are potentiated) by tamoxifen treatment (Catelli et al. 1980; Lebouc et al. 1985). Calcification of the egg shell is also not a singularly estrogenic process (Qin and Klandorf 1995), and tamoxifen does not appear to affect activity or concentration of the calcium-binding protein (calbindin) in the avian intestine or eggshell gland in laying hens (Bar et al. 1996). The specific effect of tamoxifen on egg size via the process of yolk formation is also confirmed by Williams's (2000) observation that tamoxifen treatment reduced relative yolk mass and yolk protein content but did not alter the relative proportion of yolk lipid, albumen, or shell. Our results are therefore entirely consistent with tamoxifen having specific effects on early stages of rapid yolk development via suppression of hepatic vitellogenin production; tamoxifen decreased egg size but had no effect on the timing components of egg production (laying interval or laying rate), the pattern of within-clutch variation in egg size, or, in this study, the number of eggs laid. It is notable how robust the intraclutch pattern of egg size variation is despite this tamoxifen-induced perturbation of egg formation, although the mechanisms that control this fine-scale variation in egg size remain unknown.

Increasing the frequency of tamoxifen treatment in this study (using daily injections; cf. Williams 2000, 2001) resulted in a

Table 4: Body mass and size measurements at fledging and maturity and sex ratio for adult offspring of sham-treated and tamoxifen-treated females

Trait	Sham Offspring	Tamoxifen Offspring
Fledging mass (g)**	13.22 ± .13 (30)	12.52 ± .18 (24)
Fledging tarsus length (mm)	16.69 ± .11 (29)	16.31 ± .13 (24)
Fledging bill length (mm)*	9.59 ± .09 (29)	8.88 ± .095 (24)
Adult mass (g)	16.54 ± .26 (29)	16.41 ± .30 (24)
Adult tarsus length (mm)	16.35 ± .16 (29)	16.28 ± .16 (24)
Adult bill length (mm)	10.16 ± .12 (29)	10.03 ± .13 (24)
Sex ratio at maturity	19 female : 11 male	15 female : 9 male

Note. Values are least squares means ± SEM, followed by sample size in parentheses.

* $P < 0.001$.

** $P < 0.0001$.

Table 5: Comparison of mean egg mass, clutch size, and change in mean egg mass and clutch size (compared to pretreatment values) in relation to reproductive outcome (fate of eggs and/or chicks within a clutch) for tamoxifen-treated females

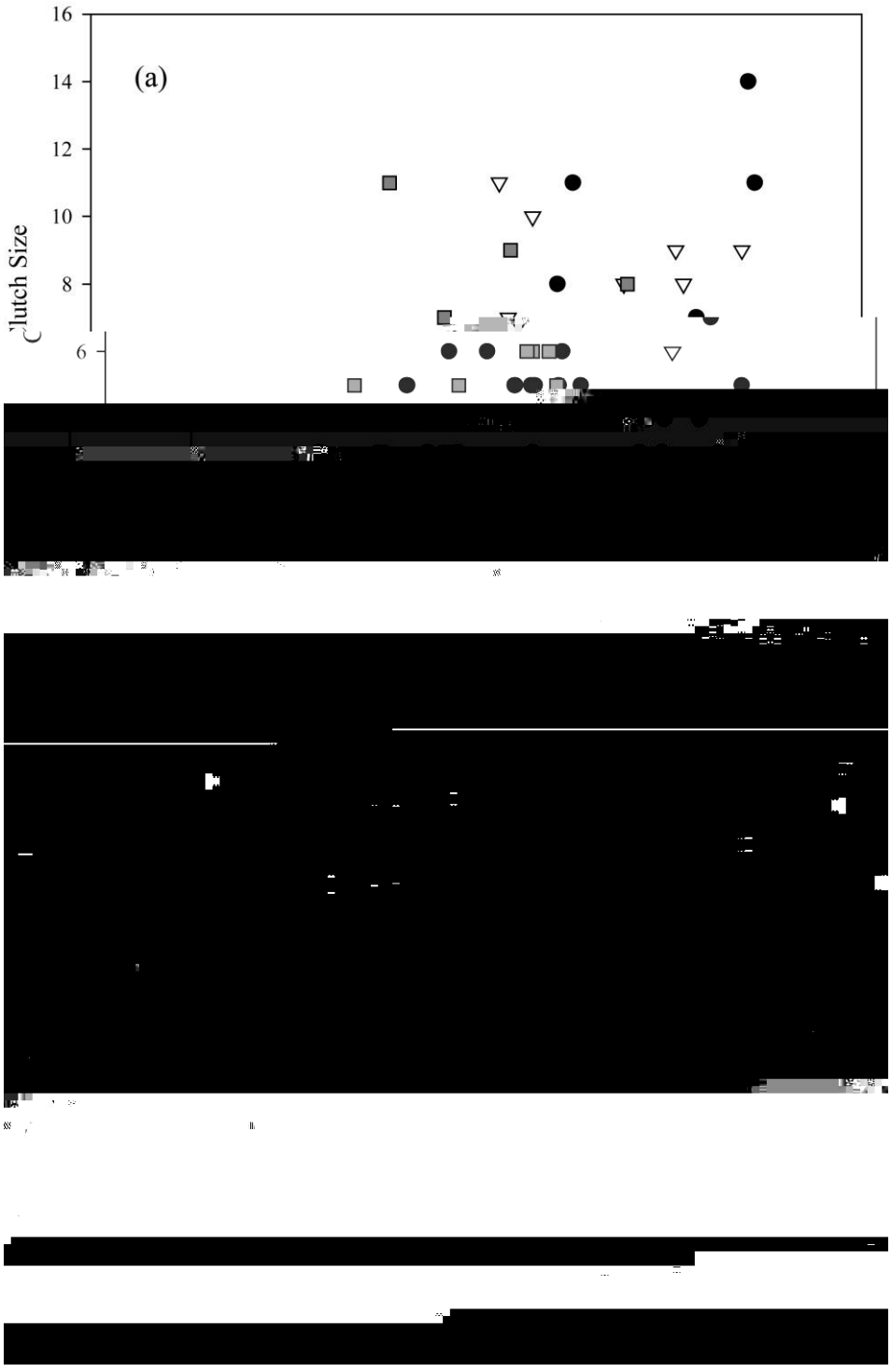

Figure 5. Relationship between reproductive outcome (i.e., the fate of eggs and/or offspring within a clutch) and (*a*) absolute clutch size and mean egg mass or (*b*) difference in clutch size and mean egg mass (relative to pretreatment values) for tamoxifen-treated females. Relative to pretreatment values, females that laid nonviable clutches (*circles*) showed decreases in both egg mass and clutch size, females that produced offspring that hatched but did not survive to maturity (*squares*) showed decreased egg mass but a larger relative clutch size, and females that produced at least one offspring that survived to fledging (*triangles*) showed the smallest relative decrease in egg mass and laid a larger clutch.

2001) and the lizard *Uta stanburiana* (Sinervo and Licht 1991; Sinervo 1999).

Despite the existence of a very high level of interindividual variability in egg size in most avian populations (Christians 2002), there is still surprisingly little empirical evidence for a strong, positive relationship between egg size and offspring fit-

Sanchez-Lafuente A.M. 2004. Trade-off between clutch size and